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The SAR analysis of TRPV1 agonists with the α-methylated B-region

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ABSTRACT

A series of TRPV1 agonists with amide, reverse amide, and thiourea groups in the B-region and their corresponding α-methylated analogues were investigated. Whereas the α-methylation of the amide B-region enhanced the binding affinities and potencies as agonists, that of the reverse amide and thiourea led to a reduction in receptor affinity. The analysis indicated that proper hydrogen bonding as well as steric effects in the B-region are critical for receptor binding.

The transient receptor potential V 1 (TRPV1) is a molecular integrator of nociceptive stimuli expressed predominantly on unmyelinated pain-sensing nerve fibers (C-fibers) and small A δ fibers in the dorsal root, trigeminal, and nodose ganglia.[1–3] TRPV1 is structurally a homotetramer and functions as a non-selective cation channel with high Ca^{2+} permeability. The receptor is activated not only by protons,[4] heat,[5] and endogenous substances such as anandamide[6] and lipoxygenase products[7] but also by natural vanilloids such as capsaicin (CAP)[8] and resiniferatoxin (RTX)[9] or indirectly by bradykinin.[10] The activation of TRPV1 by these agents leads to an increase in intracellular Ca^{2+} resulting in excitation of the primary sensory neurons. The functional blockade of this receptor, by antagonism or by desensitization subsequent to stimulation by agonists, promises considerable therapeutic utility targeting inflammatory and neuropathic pain, cystitis, and bladder hyperreflexia.[11–15]

Naturally found TRPV1 ligands, resiniferatoxin (RTX) and capsaicin (Fig. 1), have a vanilloid moiety (A-region) that is connected through either an ester linkage or amide bond (B-region) to a diterpene moiety or a long alkyl chain (C-region), respectively.[16] Recent SAR studies indicated that the modification of the A-region, especially the modification of the 4-hydroxyl group, led to a complete loss of agonistic activity,[18] and replacing the B-region linkage with a reverse amide, hydroxamate or thiourea altered the potency.[19]

Previously we have reported that the A-region derivatives of TRPV1 antagonists with an α-methyl group demonstrated enhanced binding affinity and potent antagonism for TRPV1.[17] As a continuing effort to investigate the SAR of TRPV1 agonists based on the A-, B- and C-regions, we have synthesized, based on our lead agonists (1 and 2), a series of TRPV1 agonists bearing amide, reverse amide and thiourea B-regions together with their corresponding α-methylated analogues in the B-region, and we have analyzed the effect of α-methylation on receptor affinity and agonist potency. We describe here the syntheses and functional TRPV1 activities of the designed ligands and the SAR analysis of the B-region.

The syntheses of the 3-substituted *N*-(4-*t*-butylbenzyl)-(4-hydroxyphenyl)acetamide/propionamide analogues were started from commercially available 3-substituted-4-hydroxyphenyl acetic acids **3a–c** (Scheme 1). The acetamides **4b–c** were prepared from **3b–c**, respectively, by the coupling with 4-*t*-butylbenzylamine. Dibenzylation of **3** followed by alkylation using LHMDS and methyl iodide afforded **5**. Compound **5a** was debenzylated and then coupled with the amine to provide the 3-methoxy propanamide **8a**. On the other hand, compounds **5b–c** were hydrolyzed to the acids **7** under basic conditions; the acids were then coupled with the amine and subsequently deprotected to provide the 3-halo propionamides **8b–c**, respectively.

The 4-aminoethoxy acetamides **10a–c** were prepared from 3-substituted 4-hydroxyphenyl acetamides (**1**, **4b–c**) in three steps (Scheme 2). The 2-azidoethoxy group was introduced by the alkylation of the 4-hydroxyl group with 1-bromo-2-chloroethane and the subsequent displacement reaction with sodium azide to give **9**. The azide group of **9** was reduced by either Staudinger reduction or hydrogenation to yield the final amines **10a–c**.

The 4-aminoethoxy propanamides **14a–c** were prepared from 4-hydroxy intermediates **11** in the same manner as shown in Scheme 2 (Scheme 3).

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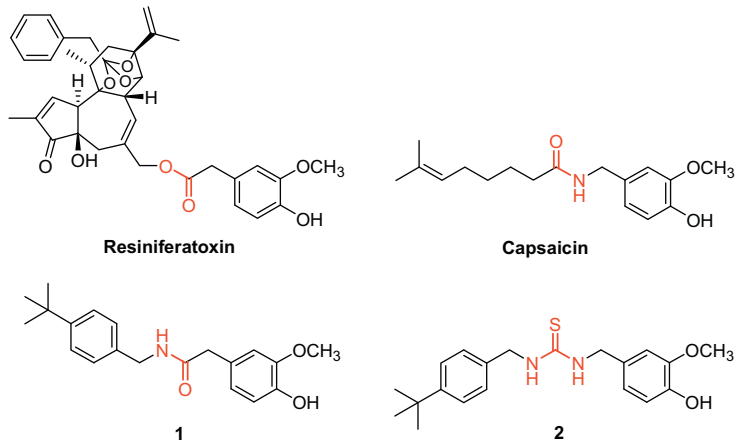
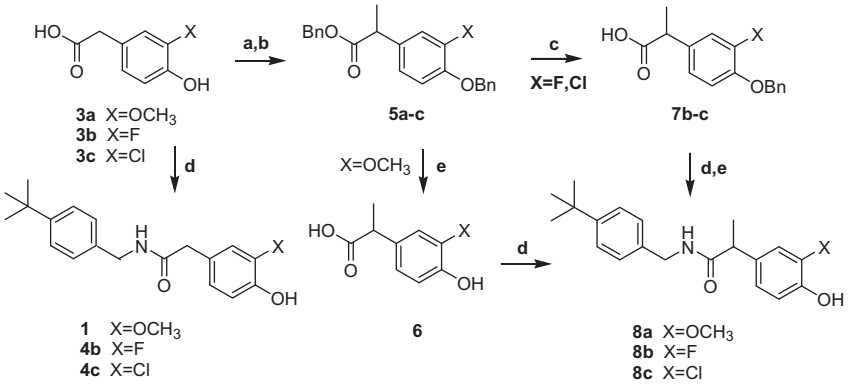
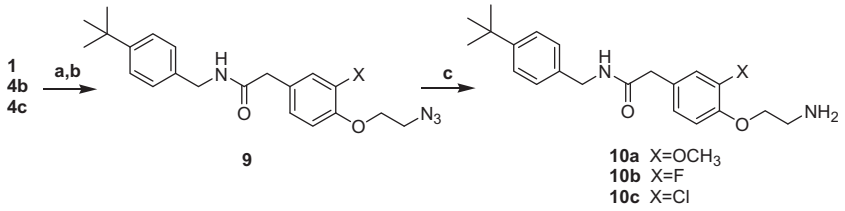


Figure 1. TRPV1 agonists.



Scheme 1. Reagents and conditions: (a) BnBr, K_2CO_3, acetone, reflux, 15 h, 98–99%; (b) LHMDS, CH_3I, THF, −78 °C, 30 min, 68–70%; (c) LiOH, THF-H_2O, rt, 15 h, 90–92%; (d) 4-*t*-butylbenzylamine, EDC, DMAP or HOBt, CH_2Cl_2, rt, 15 h, 52–80%; (e) Pd/C, H_2, CH_3OH, rt, 1 h, 70% for X = OCH_3 and 55% for X = F: 1 M BCl_3 in CH_2Cl_2, −78 °C, 62% for X = Cl.



Scheme 2. Reagents and conditions: (a) 1,2-dibromoethane, 40% KOH, Bu_4NOH, 93% for X = OCH_3: 1-bromo-2-chloroethane, K_2CO_3, acetone, reflux, 49–60% for X = F, Cl; (b) NaN_3, TBAB, KI, toluene, reflux, 71–80%; (c) PPh_3, H_2O, THF, 14% for Cl and 58% for X = OCH_3: Pd/C, H_2, CH_3OH, rt, 6 h, 38% for X = F.

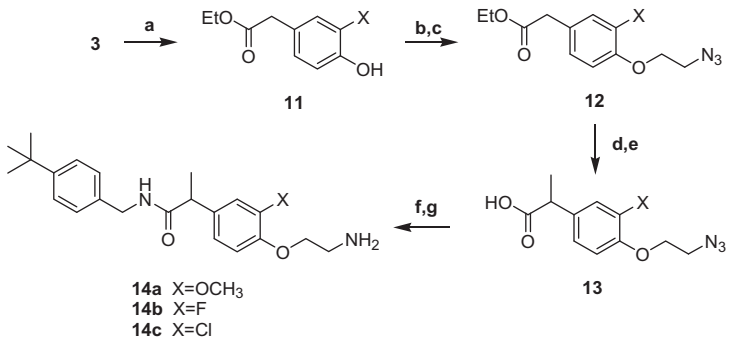
The C-region analogues of acetamide/propionamide were prepared from the acid intermediates (**3a**, **6** and **13a**) by the coupling with the corresponding amines to yield the final compounds **18–25** (Scheme 4).

The reverse amide analogues were synthesized from the benzylamine **23** or α-methyl benzylamine **28**, which was prepared from 4-hydroxy-3-methoxy acetophenone by reductive amination (Scheme 5). The coupling reactions of above benzylamines with commercially available fatty acids provided the C-region analogues with reverse amide **24–36** and α-methyl reverse amide **29–31**. α-Methyl thiourea analogue **32** was also prepared from **28** by the coupling with 4-*t*-butylbenzyl isothiocyanate.

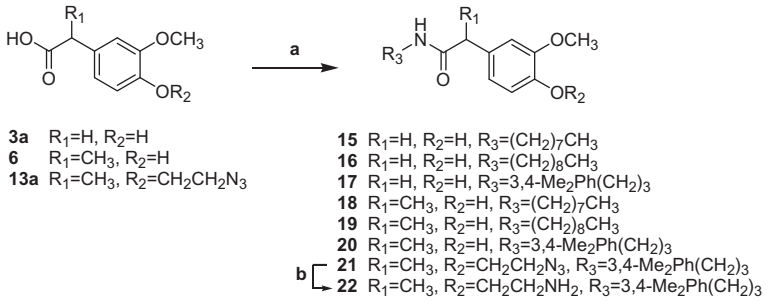
The 4-aminoethoxy thiourea **34** and its α-methyl analogue **36** were readily synthesized from the corresponding benzylamines **23** and **28** by following the routes described in Schemes 3 and 5, respectively (Scheme 6).

The binding affinities and agonistic/antagonistic functional activities of the synthesized TRPV1 ligands were determined in vitro by a binding competition assay with [^{3}H]RTX and a functional $^{45}Ca^{2+}$ uptake assay using rat TRPV1 heterologously expressed in Chinese hamster ovary (CHO) cells, as previously described.[20] The results are summarized in Tables 1–4, together with the potencies of the parent agonists **1** and **2**.

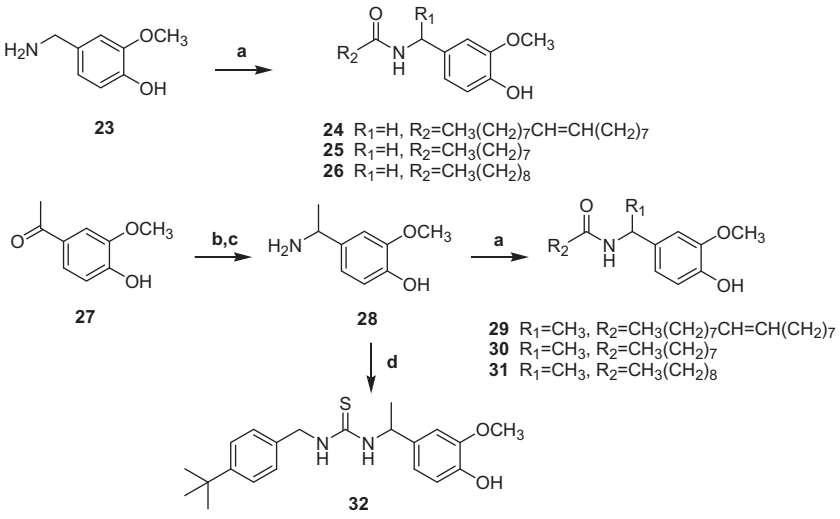
In order to investigate how the introduction of an α-methyl group into the B-region of agonists affects their TRPV1 binding and functional activity as compared to that of the parent compounds, we first fixed the C-region as a 4-*tert*-butylbenzyl moiety and varied the A-region functionality (Table 1). Compound **8a**, an α-methylated analogue of the parent agonist (**1**), showed enhanced binding affinity and agonism by 2- and 3-fold, respectively. When we replaced the 3-methoxy group with halides to provide **8b** and **8c**, these analogues bound TRPV1 even more tightly (K_i = 178 nM



Scheme 3. Reagents and conditions: (a) cat. H_2SO_4, EtOH, reflux, 98–99%; (b) 1-bromo-2-chloroethane, K_2CO_3, acetone, reflux, 55–65%; (c) NaN_3, TBAB, KI, toluene, reflux, 6 h, 90–95%; (d) NaH, CH_3I, DMF, 0 °C, 1 h, 65–75%; (e) NaOH, H_2O, reflux, 2 h, 98–99%; (f) 4-*tert*-butylbenzylamine, EDC, DMAP, CH_2Cl_2, rt, 15 h, 70–74%; (g) Pd/C, H_2, CH_3OH, rt, 80–85%.



Scheme 4. Reagents and conditions: (a) EDC, DMAP, DMF, rt, 15 h, 70–80%; (b) Pd/C, H_2, CH_3OH, rt, 80%.

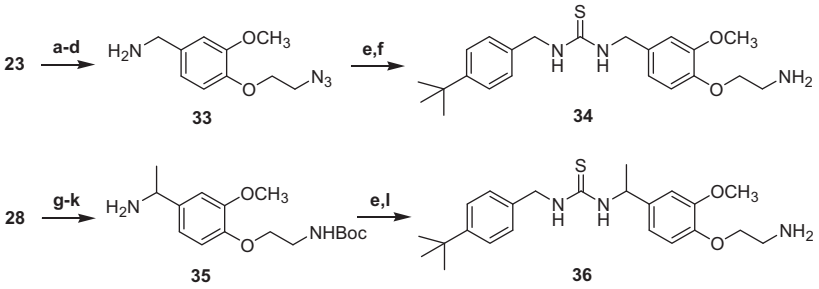


Scheme 5. Reagents and conditions: (a) RNH_2, EDC, DMAP, Et_3N, DMF, rt, 15 h, 65–70%; (b) $NH_2OH{\cdot}HCl$, Pyridine, CH_2Cl_2, reflux, 95%; (c) Pd/C, conc.·HCl, MeOH, rt, 15 h, 90%; (d) 4-t-butylbenzyl isothiocyanate, NEt_3, CH_2Cl_2, rt, 60%.

for **8b**, K_i = 33 nM for **8c**) and their affinities increased ca. 4.5- and 4-fold compared to **4b** and **4c**, respectively. However, their functional activity shifted towards partial agonism/partial antagonism (as most evident for the decreased level of agonism of **8c**). The SAR pattern in which the halogenations in the A-region shifted the functional activity to antagonism, with increased antagonism as the size of the halogen increased, was established in our previous report.[21]

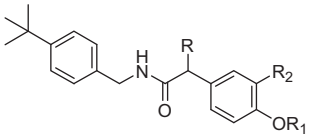
Previously, we reported that 2-aminoethylation of the 4-hydroxyl group in the capsainoid improved in vivo stability as well as analgesic activity compared to the parent agonist.[22] Thereby, we also examined the effect of α-methylation on the 2-aminoethoxy analogues of the amide agonists. Although all 4-aminoethoxy analogues synthesized were found to be partial agonists/antagonists, the binding affinities of α-methylated analogues **14a–c** increased significantly compared to the acetamides **10a–c** by 2.5- to 6.5-fold, respectively.

Next, we swapped the 4-*tert*-butylbenzyl group of **1** with long alkyl and arylalkyl chains, resembling the C-region of capsaicin, to provide **15**–**17** and examined the effect of their α-methylation (**Table 2**). Whereas the α-methylation of acetamide **15** did not affect its receptor activity, α-methylated analogues **19** and **20**



Scheme 6. Reagents and conditions: (a) $(Boc)_2O$, NEt_3, H_2O-Dioxane, rt, 2 h, 99%; (b) 1,2-dibromoethane, 40% KOH, Bu_4NOH, 50 °C, 2 h, 90%; (c) NaN_3, DMF, 100 °C, 15 h, 74%; (d) TFA,CH_2Cl_2, 0 °C, 5 h; (e) 4-*tert*-butylbenzyl isothiocyanate, NEt_3, CH_2Cl_2, rt, 15 h, 60%; (f) PPh_3, H_2O, THF, 99%; (g) 1-bromo-2-chloroethane, K_2CO_3, acetone, reflux, 3 h, 94%; (h) NaN_3, TBAB, KI, toluene, reflux, 99%; (i) $(Boc)_2O$, Pd/C, H_2, EtOH, rt; (j) $NH_2OH{\cdot}HCl$, pyridine, 80 °C, 2 h; (k) Pd/C, H_2, conc. HCl, EtOH, rt, 15 h; (l) TFA, CH_2Cl_2, 0 °C, 1 h, 55%; PPh_3, H_2O, THF, 99%.

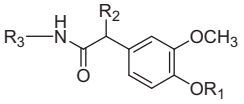
Table 1
Amide B-region with 4-*t*-butylbenzyl C-region



	R	R_1	R_2	K_i (nM) binding affinity	EC_{50} (nM) agonism	K_i (nM) antagonism
1	H	H	OCH_3	667 (±86)	82 (±34)	NE
4b	H	H	F	808 (±179)	911 (±88)	(30%)[b]
4c	H	H	Cl	132 (±29)	206 (±41)	NE
8a	CH_3	H	OCH_3	308 (±7.0)	24 (±6.4)	(15%)[b]
8b	CH_3	H	F	178 (±33)	221 (±63)	(20%)[b]
8c	CH_3	H	Cl	33.1 (±6.6)	(77%)[a]	(9%)[b]
10a	H	$CH_2CH_2NH_2$	OCH_3	1230 (±230)	358 (±58)	(12%)[b]
10b	H	$CH_2CH_2NH_2$	F	10,100 (±1300)	(63%)[a]	(18%)[b]
10c	H	$CH_2CH_2NH_2$	Cl	2470 (±490)	(36%)[a]	(56%)[b]
14a	CH_3	$CH_2CH_2NH_2$	OCH_3	504 (±160)	238 (±79)	(16%)[b]
14b	CH_3	$CH_2CH_2NH_2$	F	2160 (±410)	(91%)[a]	NE
14c	CH_3	$CH_2CH_2NH_2$	Cl	380 (±110)	(75%)[a]	(38%)[b]

[A] Only fractional calcium uptake compared with that induced by 300 nM capsaicin.
[B] Only fractional antagonism at 30 μM.

Table 2
Amide B-region with alkyl and arylalkyl C-region

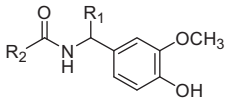


	R_1	R_2	R_3	K_i (nM) binding affinity	EC_{50} (nM) agonism	K_i (nM) antagonism
15	H	H	$(CH_2)_7CH_3$	848 (±67)	77 (±18)	NE
16	H	H	$(CH_2)_8CH_3$	240 (±43)	61 (±16)	NE
17	H	H	$(CH_2)_3Ph(3,4\text{-}Me_2)$	354 (±84)	41 (±26)	NE
18	H	CH_3	$(CH_2)_7CH_3$	744 (±32)	130 (±16)	NE
19	H	CH_3	$(CH_2)_8CH_3$	159 (±22)	26.3 (±7.6)	NE
20	H	CH_3	$(CH_2)_3Ph(3,4\text{-}Me_2)$	177 (±30)	21.3 (±2.7)	NE
DA-5018	$CH_2CH_2NH_2$	H	$(CH_2)_3Ph(3,4\text{-}Me_2)$	930 (±170)	589 (±76)	NE
22	$CH_2CH_2NH_2$	CH_3	$(CH_2)_3Ph(3,4\text{-}Me_2)$	518 (±110)	404 (±67)	NE

showed a 1.5- to 2-fold enhancement in binding affinity and agonism compared to **16** (nonivamide) and **17**. DA-5018 is the 2-aminoethyl analogue of **17**. The α-methylation of DA-5018 to generate **22** likewise led to ca. 2-fold increase in receptor activity. The results indicated that α-methylation in the amide B-region might enhance favorable hydrogen bonding with the receptor or provide an additional hydrophobic interaction of a methyl group with the receptor.

To further evaluate the effect of α-methylation in the B-region, we also investigated the reverse amide (Table 3) and thiourea B-regions (Table 4) in a similar fashion. The reverse amide B-region agonists olvanil **24** and **25**–**26** and their α-methylated analogues **29**–**30** were prepared for the comparison of receptor activities. The analysis indicated that α-methylation in the reverse amide B-region led to a decrease in receptor activity in which **29–31** showed 2- to 4- fold reductions in binding affinity compared to

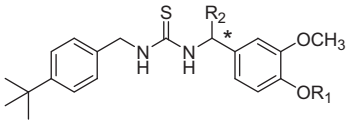
Table 3
Reverse amide B-region with alkyl C-region



	R_1	R_2	K_i (nM) binding affinity	EC_{50} (nM) agonism	K_i (nM) antagonism
24 (Olvanil)	H	$(CH_2)_7CH{=}CH(CH_2)_7CH_3$	142 (±27)	202 (±19)	NE
25	H	$(CH_2)_7CH_3$	1223 (±18)	223 (±47)	NE
26	H	$(CH_2)_8CH_3$	864 (±10)	83 (±16)	NE
29	CH_3	$(CH_2)_7CH{=}CH(CH_2)_7CH_3$	226 (±52)	678 (±140)	NE
30	CH_3	$(CH_2)_7CH_3$	4275 (±99)	1580 (±290)[a]	(44%)[b]
31	CH_3	$(CH_2)_8CH_3$	3300 (±610)	1250 (±140)	(28%)[b]

[A] Only fractional calcium uptake (72%) compared with that induced by 300 nM capsaicin.
[B] Only fractional antagonism at 30 μM.

Table 4
Thiourea B-region with 4-*t*-butylbenzyl C-region



	R_1	R_2	K_i (nM) binding affinity	EC_{50} (nM) agonism	K_i (nM) antagonism
2	H	H	59 (±9.0)	2.6 (±1.1)	NE
32	H	CH_3	194 (±5)	380 (±160)[a]	(35%)[b]
34	$CH_2CH_2NH_2$	H	84 (±27)	132 (±34)	NE
36	$CH_2CH_2NH_2$	CH_3	1430 (±520)	5800 (±1700)	NE

[A] Only fractional calcium uptake (70%) compared with that induced by 300 nM capsaicin.
[B] Only fractional antagonism at 30 μM.

the corresponding acetamides. The similar SAR pattern was also observed in the thiourea B-region. The α-methylated thioureas **32** and **36** showed marked loss of binding affinity compared to **2** and **34** by 3- and 17-fold. The result indicated that, different from the amide B-region, the α-methylation of the reverse amide and thiourea might interfere with appropriate hydrogen bonding with the receptor due to steric hindrance.

In summary, we have synthesized a series of TRPV1 agonists with the amide, reverse amide, and thiourea groups in the B-region along with their corresponding α-methylated analogues. Within the amide analogues, the α-methylation enhanced binding affinity and agonism compared to the parent agonist due to favorable hydrogen bonding interaction or additional hydrophobic interaction of a methyl in the binding site. By contrast, the α-methylation of the reverse amide and thiourea agonists led to the reduction in receptor activity, suggesting that the placement of a methyl group might interfere with appropriate hydrogen bonding with the receptor due to steric hindrance. The SAR analysis of α-methylation implicates hydrogen bonding as well as a steric effect in the B-region as critical for receptor binding. The SAR of the two enantiomers of the α-methylated amide is underway to further explore interactions with the receptor.

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